Ryanodine Receptors Are Expressed in Epidermal Keratinocytes and Associated with Keratinocyte Differentiation and Epidermal Permeability Barrier Homeostasis

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Ryanodine receptors (RyRs) have an important role as calcium channels in the regulation of intracellular calcium levels in the nervous system and muscle. In the present study, we investigated the expression of RyR in human epidermis. Immunohistochemical studies and reverse transcription-PCR indicated the expression of RyR type 1, 2, and 3 proteins in epidermal keratinocytes. The expression level of each RyR subtype was higher in differentiating keratinocytes than in proliferative cells. We also demonstrated the functional expression of RyR by calcium imaging. In cultured human keratinocytes, application of the RyR agonist 4-chloro-*m*-cresol (CMC) induced elevation of the intracellular calcium concentration, and co-application of the RyR antagonist 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHBP) blocked the elevation. Application of CMC accelerated keratinocyte differentiation *in vitro*. On the other hand, topical application of CMC after tape-stripping of hairless mouse skin delayed barrier recovery, whereas application of an RyR antagonist, dantrolene or DHBP, accelerated the barrier recovery. These results suggest that RyR expressed in epidermal keratinocytes is associated with both differentiation of keratinocytes and epidermal barrier homeostasis.

Journal of Investigative Dermatology (2012) **132,** 69–75; doi:10.1038/jid.2011.256; published online 1 September 2011

INTRODUCTION

Calcium dynamics in epidermal keratinocytes has key roles in keratinocyte differentiation and water-impermeable barrier homeostasis. We have previously shown that various neurotransmitter receptors are expressed in epidermal keratinocytes (Denda *et al.*, 2007b). Activation of excitatory receptors, such as *N*-methyl-D-aspartate receptor, nicotinic acetylcholine receptor, and P2X3 receptor, induces calcium influx and delays barrier recovery (Denda *et al.*, 2002, 2003a; Fuziwara *et al.*, 2003). Application of a calcium ionophore, which also induces calcium influx, also delayed barrier recovery (Denda *et al.*, 2003a). These results suggest that elevation of intracellular calcium concentration in epidermal keratinocytes delays barrier recovery.

The excitatory receptors mentioned above are expressed in the cell membrane and serve to regulate the intracellular calcium level. On the other hand, the endoplasmic reticulum (ER) also has an important role as a calcium store and regulates calcium signaling in many types of cells. IP3 receptor and ryanodine receptor (RyR) are the major calcium channels mediating calcium-induced calcium release (Berridge *et al.*, 2000).

It has been shown that IP3 receptor is associated with TRPV3 signaling in mouse keratinocytes (Cheng *et al.*, 2010). We recently demonstrated that IP3 had an important role in calcium propagation in cultured human keratinocytes (Tsutsumi *et al.*, 2010). RyR is expressed in the ER or sarcoplasmic reticulum in muscle. Three RyR subtypes have been described, and many cell types are known to express each of them. Their roles have only been extensively characterized in tissues in which they are abundant: RyR1 is essential for excitation–contraction coupling in the skeletal muscle, whereas RyR2 is essential in the cardiac muscle. RyR2 and RyR3 have roles in intracellular calcium regulation in the brain (Zucchi and Ronca-Testoni, 1997; Kushnir *et al.*, 2010). As mentioned above, there are similarities of receptor expression between neurons and keratinocytes, both of which are derived from the ectoderm during the early stage of development. Thus, we hypothesized that RyR might be expressed in epidermal keratinocytes and be associated with cellular differentiation and/or epidermal permeability barrier homeostasis.

In the present study, we first examined whether or not RyR is present in epidermal keratinocytes by means of

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Abbreviations: CMC, 4-chloro-m-cresol; DHBP, 1,1′-diheptyl-4,4′-bipyridinium dibromide; ER, endoplasmic reticulum; RyR, ryanodine receptor

Received 3 February 2011; revised 9 June 2011; accepted 8 July 2011; published online 1 September 2011

immunohistochemistry and reverse transcription-PCR. Then, we evaluated the response of intracellular calcium to RyR modulators. We also examined the effects of these modulators on keratinocyte differentiation. Finally, we studied the effects of topical application of these modulators on the recovery process after barrier disruption.

RESULTS

Immunoreactivity to RyR antibodies was observed in the epidermis of human skin (Figure 1). RyR1 was expressed strongly in the differentiated layers of the epidermis. RyR2 was also expressed in the differentiated layers, especially in the border layer between the stratum corneum and the granular layer. RyR3 was expressed throughout the epidermis, but the expression was stronger in the differentiated layers than in the basal layer. Conventional reverse transcription-PCR analysis showed that mRNAs for all RyR subtypes, including the two alternative splice variants of RyR3, were present in human skin and cultured epidermal keratinocytes (Figure 2). Quantitative PCR analysis revealed that expression of all RyR subtypes in cultured keratinocytes was dramatically increased after the induction of differentiation by high Ca^{2+} (Figure 3). This result is consistent with the characteristic distribution *in vivo* (Figure 1).

Functional activity of RyRs in keratinocytes was confirmed by studying changes in intracellular free calcium concentration ($[Ca^{2+}]_i$) in response to RyR modulators (Figure 4). In a Ca^{2+}-free bath solution, application of an RyR agonist, 4-chloro-*m*-cresol (CMC; Ducreux *et al.*, 2004), induced elevation of $[Ca^{2+}]_i$ (Figure 4a), and the elevation was inhibited by an RyR antagonist, 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHBP; Lauckner *et al.*, 2005; Figure 4b, second stimulation). The CMC response disappeared when intracellular Ca^{2+} was chelated with BAPTA-AM (Figure 4c). Although the CMC response, expressed as change of fluorescence ratio, was smaller than the ionomycin response (Figure 4e), it was comparable with the thapsigargin response (Figure 4d). Thapsigargin releases Ca^{2+} from the ER store by

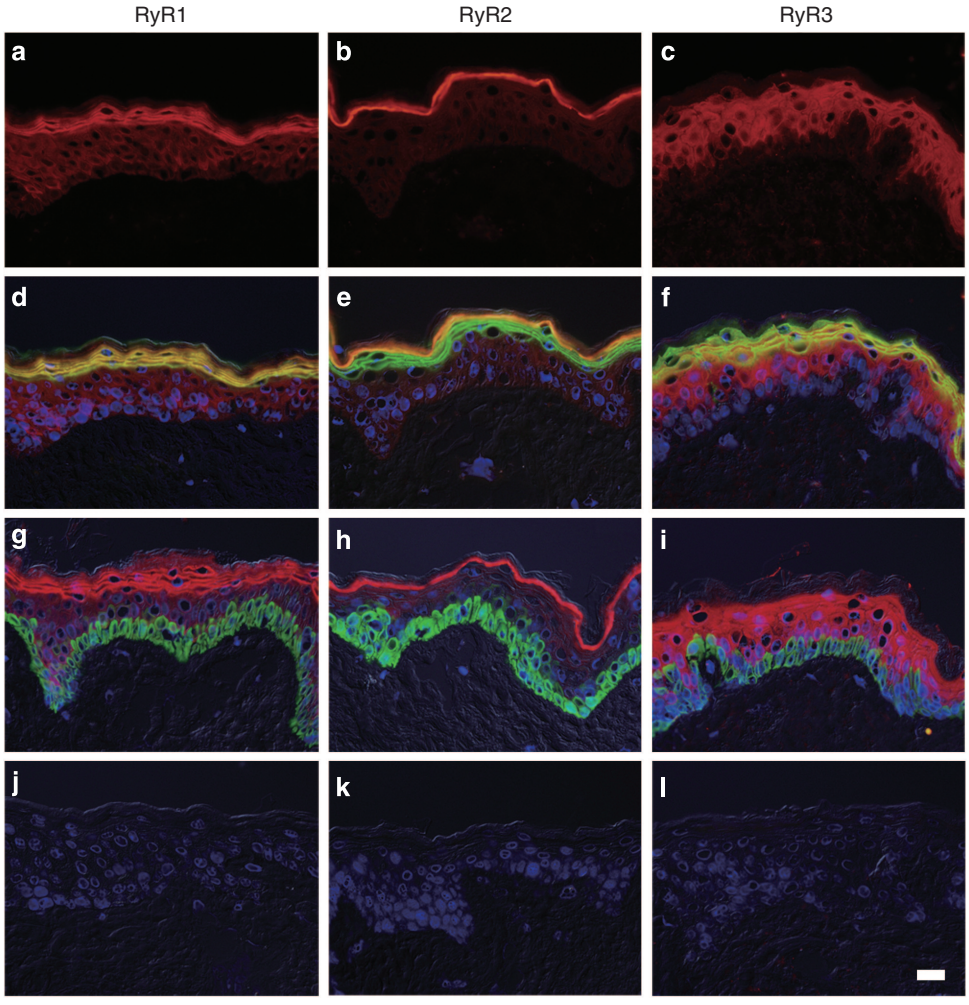

Figure 1. Immunohistochemical study of human skin with ryanodine receptor (RyR) antibodies. (**a**, **d**, **g**) Anti-RyR1 (red); (**b**, **e**, **h**) anti-RyR2 (red); (**c**, **f**, **i**) anti-RyR3 (red); (**d**, **e**, **f**) merged with anti-involucrin (green) and differential interference contrast image to observe the unstained stratum corneum; (**g**, **h**, **i**) merged with anti-keratin 14 (green) to observe the basal layer of the epidermis; (**j**, **k**, **l**) without primary antibodies. RyR was observed in the epidermis with distinct subtype distribution patterns. Bar = 20 μm.

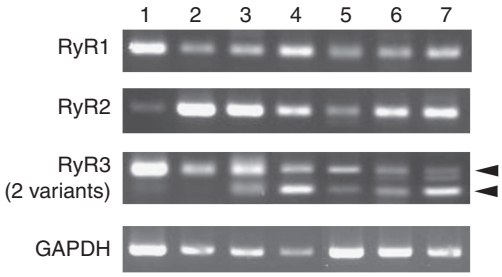

Figure 2. Detection of ryanodine receptor (RyR) expression by reverse transcription-PCR. Lane 1, skeletal muscle (positive control for RyR1); lane 2, heart (positive control for RyR2); lane 3, brain (positive control for all RyRs); lane 4, skin; lane 5, proliferative keratinocytes; lane 6, differentiating keratinocytes (2 days after induction); and lane 7, differentiating keratinocytes (4 days after induction). The PCR product sizes for RyR1 and RyR2 were 251 and 290 bp, respectively. The PCR for RyR3 detected two alternative splice variants (152 and 234 bp), as reported (Klegeris *et al.*, 2007). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

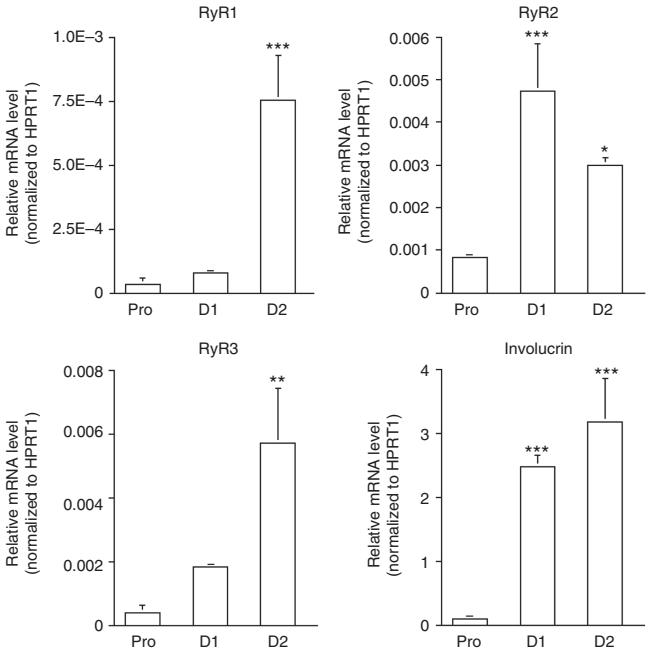

Figure 3. Changes in the expression of ryanodine receptor (RyR) subtype mRNAs in differentiating keratinocytes. RyR mRNA levels were measured by quantitative PCR. Expression levels of each subtype of RyR and involucrin (as a differentiation marker) were increased in differentiating keratinocytes. Asterisks denote statistical significance as compared with Pro ($n=3$). *$P<0.05$, **$P<0.01$, ***$P<0.001$. D1, differentiating keratinocytes (1 day after induction); D2, differentiating keratinocytes (2 days after induction); HPRT1, hypoxanthine phosphoribosyl transferase 1; Pro, proliferative keratinocytes.

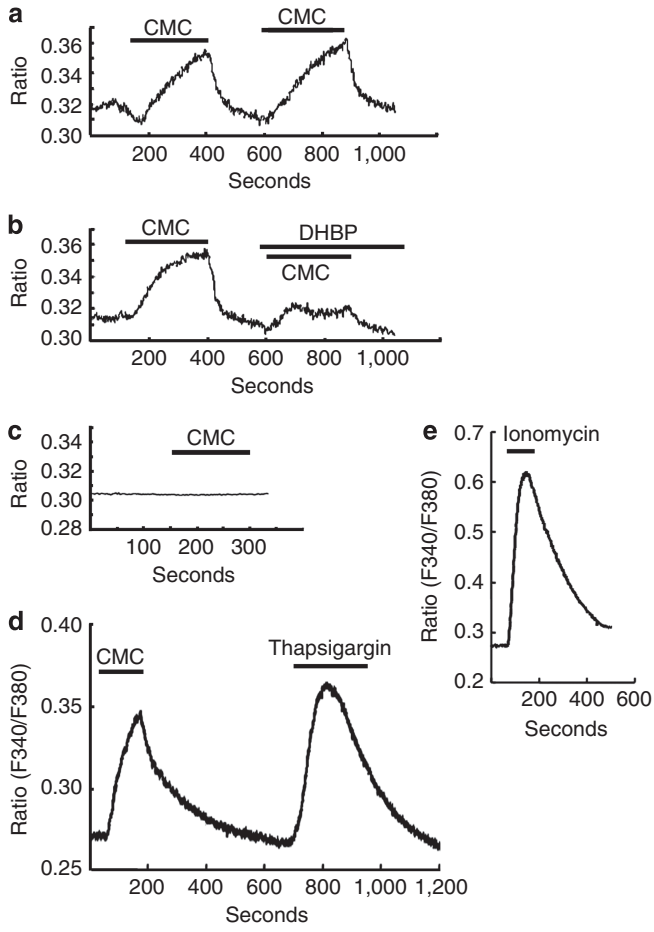

Figure 4. Calcium imaging of keratinocytes. Normal human epidermal keratinocytes were treated with the indicated reagents, in a Ca^{2+}-free (**a**–**d**) or 0.06 mM Ca^{2+}-containing (**e**) bath solution. Almost 100% of the cells in the microscopic field responded to the reagents. Representative profiles of fura-2 ratio (F340/F380) in a single cell are shown. Experiments were repeated three times with similar results. (**a**) A ryanodine receptor (RyR) agonist, 4-chloro-*m*-cresol (CMC, 0.5 mM), was applied twice, each for 3 minutes. (**b**) For the second CMC stimulation, a RyR antagonist, 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHBP, 100 μM), was also applied. (**c**) Cells were preincubated for 30 minutes with 50 μM BAPTA-AM in the medium, and then CMC was applied. (**d**) CMC was applied first, and then thapsigargin (100 nM) was applied to observe the response when Ca^{2+} is released from the endoplasmic reticulum store. (**e**) Ionomycin (10 μM) was applied to obtain the maximal response. Application of CMC induced the elevation of $[Ca^{2+}]_i$, and this action was inhibited by DHBP. These results show that RyR is functional in keratinocytes.

inhibiting sarco/endoplasmic Ca^{2+}-ATPase (Thastrup *et al.*, 1990). Therefore, the elevation of $[Ca^{2+}]_i$ by CMC appears to depend on Ca^{2+} release from the intracellular calcium store, via the opening of the RyR channel.

As the abundance of RyR in differentiated keratinocytes suggests a role in epidermal differentiation, we next studied the effects of the RyR agonist CMC on the expression of differentiation marker genes in keratinocytes (Figure 5a and b). Involucrin is a component of cornified envelope (CE), which is the product of terminal differentiation of keratinocytes, and transglutaminase 3 is specifically expressed in the granular layers of the epidermis, where it is activated by $[Ca^{2+}]_i$ elevation to function for CE formation (Candi *et al.*, 2005). In undifferentiated keratinocytes, CMC increased the mRNA expression of both genes. In keratinocytes, just after the induction of differentiation with high Ca^{2+}, the RyR antagonists DHBP and dantrolene (Mackrill, 2010) both inhibited involucrin expression (Figure 5c). The promoting

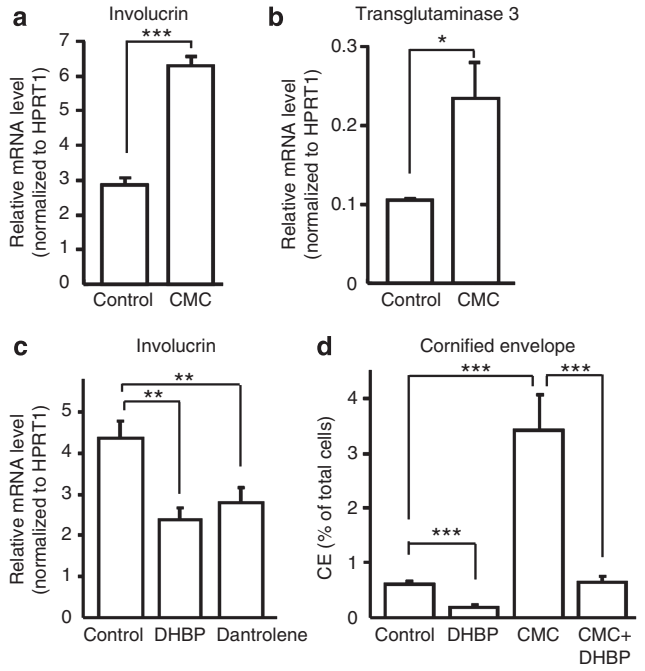

Figure 5. Effects of ryanodine receptor (RyR) modulators on keratinocyte differentiation. (**a**, **b**, **c**) Expression levels of differentiation marker genes were measured by quantitative PCR after 24 hours incubation. (**a**, **b**) Undifferentiated confluent keratinocytes were treated with 4-chloro-*m*-cresol (CMC; 0.3 mM) in medium containing 0.06 mM $CaCl_2$. CMC increased the mRNA expression of involucrin and transglutaminase 3. (**c**) Confluent keratinocytes were treated with 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHBP; 45 μM) or dantrolene (45 μM), at the same time as induction of differentiation with high Ca^{2+}. Both RyR antagonists inhibited involucrin expression. (**d**) Cornified envelope (CE), the product of terminal differentiation of keratinocytes, was counted after a 4-day incubation with the reagents. The total cell number at harvest was 2–4 × 10^6 cells per well. The CE was increased in the presence of CMC (0.3 mM), and the increase was abolished when DHBP (15 μM) was co-applied with CMC. DHBP alone inhibited the CE formation. Asterisks denote statistical significance ($n=3$). *$P<0.05$, **$P<0.01$, ***$P<0.001$. HPRT1, hypoxanthine phosphoribosyl transferase 1.

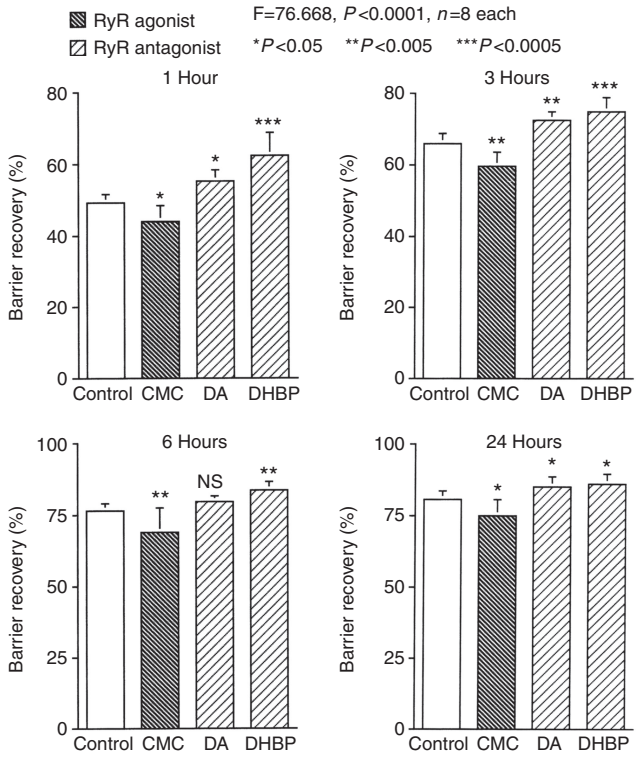

Figure 6. Effects of topical application of ryanodine receptor (RyR) modulators on the barrier recovery rate of the skin. A RyR agonist, 4-chloro-*m*-cresol (CMC; 2.5 mM), delayed the barrier recovery after tape-stripping, whereas RyR antagonists, dantrolene (DA; 100 μM) and 1,1′-diheptyl-4,4′-bipyridinium dibromide (DHBP; 250 μM), accelerated the recovery. NS, not significant.

activity of CMC and inhibiting activity of DHBP on cellular differentiation were further supported by analysis of CE (Figure 5d). In the presence of high Ca^{2+}, the percentage of cells containing CE was increased after incubation with CMC, but this increase was blocked when DHBP was co-applied with CMC. DHBP alone inhibited CE formation. These results indicate that the elevation of $[Ca^{2+}]_i$ by the RyR agonist may induce the synthesis of mRNAs and proteins for CE constituents, and also activate enzymes, such as transglutaminase 3, necessary for CE formation. As RyR antagonists inhibited differentiation induced by high Ca^{2+}, RyR may be involved in Ca^{2+} signal amplification and/or homeostatis after the elevation of extracellular Ca^{2+}.

The effects of topical application of RyR agonist and antagonists on the barrier recovery rate after barrier disruption are shown in Figure 6. Application of the agonist CMC significantly delayed barrier recovery, whereas application of an antagonist, dantrolene or DHBP, accelerated barrier recovery.

DISCUSSION

We previously reported that activation of calcium-permeable channels on the keratinocyte cell membrane delayed the recovery after barrier disruption (Fuziwara *et al.*, 2003; Denda *et al.*, 2003a). Moreover, activation of some G protein-coupled receptors, such as adrenergic receptors, also induced the elevation of intracellular calcium via voltage-gated calcium channels and delayed the barrier recovery (Denda *et al.*, 2003b; Denda *et al.*, 2004; Fuziwara *et al.*, 2005). That is, activation of excitatory receptors in keratinocytes delayed the barrier recovery. In these cases, intracellular calcium elevation is induced by influx of calcium ions from outside the cells.

In nerve cells or muscle cells, excitation (i.e., depolarization) is also induced by calcium ion release from the ER. However, there are few reports on the role of IP3 receptors and no report on the function of RyRs in epidermal keratinocytes. In the present study, we have demonstrated the presence of RyRs in keratinocytes for the first time, and examined their roles in epidermal differentiation and epidermal permeability barrier homeostasis.

We detected all of the three known types of RyR, although we did not clarify which type(s) of RyR were associated with keratinocyte differentiation and barrier homeostasis. The mRNA of each receptor increased with keratinocyte differentiation (Figure 3). As RyR1 and RyR2 were localized at the

uppermost layer of the epidermis (Figure 1), they may have a role in barrier homeostasis. To our knowledge, among RyR gene-targeted mice, only RyR1^{I4895T} homozygous mutant mice are reported to have a phenotype in skin. They die perinatally because of asphyxia, and the skin of the neonates exhibits a marked delay in dermatogenesis, showing a severely underdeveloped dermis with a very thin layer of relatively undifferentiated epidermis and poorly defined hair follicles (Zvaritch *et al.*, 2007). The RyR1-mediated Ca^{2+} release might also be important for skin development.

Calcium release from the ER induces calcium waves or calcium oscillations (Berridge *et al.*, 2000). We previously demonstrated that air exposure of cultured human keratinocytes also induced calcium waves and calcium oscillations (Denda and Denda, 2007a). RyR in the keratinocytes might be associated with these calcium dynamics in the epidermis. There is a calcium gradient in the epidermis (Mauro, 1998; Denda *et al.*, 2000a), with the highest level of calcium ion in the uppermost layer. Immediately after barrier insult, the gradient disappears, and with recovery of the barrier function the calcium gradient also recovers. Occlusion with a water-impermeable membrane perturbs the barrier recovery and the calcium gradient is not restored (Menon *et al.*, 1994). Thus, calcium dynamics in the epidermis seems to have a central role in barrier homeostasis. The calcium gradient is also significantly related to keratinocyte differentiation in the epidermis (Elias *et al.*, 2002). Recently, Celli *et al.* (2010) showed that barrier disruption by tape-stripping resulted in the activation of ER-stress-induced transcription factor XBP1. Our findings suggest that RyR in the epidermis might have an important role in these processes.

In the brain, RyR has a role in the secretion of oxytocin (Jin *et al.*, 2007). We previously demonstrated that various neurotransmitters, such as ATP, glutamate, and dopamine, were released from the epidermis after barrier insult (Denda *et al.*, 2002; Fuziwara *et al.*, 2003, 2005). Therefore, RyR might be associated with the secretion of these molecules.

In the present study, we have demonstrated the functional existence of RyR in epidermal keratinocytes, and we have shown that RyR modulators influence keratinocyte differentiation and barrier homeostasis. Although the mechanism of calcium-induced calcium release by RyR in keratinocytes remains to be clarified, RyR could be an new target for research on dermatological diseases.

MATERIALS AND METHODS

Materials

CMC, DHBP, and dantrolene sodium salt were purchased from Sigma-Aldrich (St Louis, MO). Human tissue was obtained following plastic surgery, after informed consent had been obtained. This study was approved by the Ethics Committee of Shiseido Research Center in accordance with the guideline of the National Institute of Health, and was conducted according to the Declaration of Helsinki Principles.

Immunohistochemistry

Frozen sections of human skin were fixed with cold methanol and reacted with specific antibodies at 4 °C overnight, then visualized with Alexa 594- or 488-conjugated secondary antibodies (Invitrogen, Carlsbad, CA). The sections were counter-stained with Hoechst 33258 for nuclear staining. The following antibodies were used: anti-RyR1 (Millipore, Billerica, MA, no. AB9078, rabbit polyclonal, at 1:2,000 dilution), anti-RyR2 (Millipore, no. AB9080, rabbit polyclonal, 1:1,000), anti-RyR3 (Santa Cruz Biotechnology, Santa Cruz, CA, no. sc-21330, goat polyclonal, 1:50), anti-keratin 14 (Millipore, no. MAB3232, 1:2,000), anti-involucrin (Novocastra/Leica, Wetzlar, Germany, clone SY5, 1:800). The subtype-specific characteristics of these RyR antibodies have been reported (Dabertrand *et al.*, 2007; Medina-Ortiz *et al.*, 2007).

Culture of keratinocytes

Normal human epidermal keratinocytes (NHEKs) were purchased from Kurabo (Osaka, Japan). NHEKs were cultured in Epilife-KG2 medium (Kurabo) containing 0.06 mM $CaCl_2$. NHEKs (passage 3–4) were seeded onto collagen-coated glass coverslips and used for calcium imaging at 60–80% confluency. For RNA preparation, cells were grown in six-well plates and harvested in the proliferative state (60–80% confluency) or the differentiated state. Cell differentiation was induced at 100% confluency by increasing the calcium concentration to 1.8 mM in the medium (Kolly *et al.*, 2005), and differentiated cells were harvested 1–4 days after the induction.

Reverse transcription-PCR

Total RNA was isolated from human keratinocytes using EZ1 RNA cell mini kit (Qiagen GmbH, Hilden, Germany), then 1 μg of total RNA was reverse-transcribed with Superscript III (Invitrogen) in a final volume of 20 μl. Human skin complementary DNA was purchased from BioChain (Hayward, CA). Human complementary DNAs from brain, skeletal muscle, and heart were purchased from Takara Bio (Otsu, Japan). Conventional PCR was performed using the RyR type-specific primers (Klegeris *et al.*, 2007). PCR reaction was performed for 38 cycles, with annealing at 55 °C. PCR for *GAPDH* gene (forward 5′-CCCATCACCATCTTCCAG-3′, reverse 5′-CCTGCTTCACCACCTTCT-3′, 577 bp product) was performed as a loading control. RyR mRNAs were measured by real-time quantitative PCR using Universal Probe Library (UPL) probes and primers designed by UPL Assay Design software, in the LightCycler 480 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The following primers and UPL probes were used: human hypoxanthine phosphoribosyl transferase 1 forward 5′-TGACCTTGATTTATTTTGCATACC-3′, reverse 5′-CGAGCAAGACGTTCAGTCCT-3′, UPL 73; RyR1 forward 5′-CCTGTGCTCAAGCTCGTGT-3′, reverse 5′-CACATCCTCATCGCCAAAG-3′, UPL 9; RyR2 forward 5′-GATGAAACAGAACACACAGGACA-3′, reverse 5′-TTTAGCTGGTCTTCATACTGTTTCC-3′, UPL 3; RyR3 forward 5′-CCTGGATGATGCTTCAATGAT-3′, reverse 5′-ATAGACCCAGACAGCAGATGG-3′, UPL 24; involucrin forward 5′-GGAGAAGCAGGAGGCACA-3′, reverse 5′-TCCAGGTGCTTTGGCTGT-3′, UPL 62; transglutaminase 3 forward 5′-ATTGGCTGGAACTTTGGACA-3′, reverse 5′-GGCGGAAATTCAGACTCCTA-3′, UPL 34. Hypoxanthine phosphoribosyl transferase 1 gene expression level was used for data normalization. Quantification of gene expression was carried out by the comparative Ct method.

Ca^{2+} imaging in single keratinocytes

Cells were incubated with 5 μM fura-2, AM (Invitrogen) for 1 hour at 37 °C. The fura-2-loaded cells were placed on the stage of an

inverted fluorescence microscope (Ti, Nikon) and perfused with Ca^{2+}-free bath solution (150 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM HEPES (4-(2-hyroxyethyl)-1-piperazine ethane sulfonic acid), 1 mM EGTA, and 10 mM D-glucose (pH 7.4)). Image capture and processing were performed using an ORCA-R2 CCD camera and Aquacosmos software (Hamamatsu Photonics, Hamamatsu, Japan). With alternating illumination at 340 and 380 nm for excitation, pairs of fluorescence images were obtained every 2 seconds through an objective lens (S Fluor ×10/0.50 numerical aperture, Nikon) and an emission filter (470–550 nm). Changes in $[Ca^{2+}]_i$ of single cells were displayed as fura-2 ratio (F340/F380).

Analysis of cornified envelope

Keratinocytes were grown in six-well plates. At 100% confluency, the cells were treated with reagents in a medium containing 1.8 mM $CaCl_2$, and incubation was continued for 4 days. The medium containing the reagent was changed every day. Then the cells were trypsinized and counted. CEs were prepared as described (Sun and Green, 1976). Briefly, a known number of trypsinized cells was resuspended in a buffer consisting of 10 mM Tris–HCl, pH 7.5, 1% 2-mercaptoethanol, and 1% SDS, and incubated at 80 °C until the plasma membrane was dissolved. The remaining CEs were counted using a hemocytometer.

Barrier recovery

Permeability barrier function was evaluated by measurement of transepidermal water loss (TEWL) with an electric moisture analyzer (Meeco, Warrington, PA), as described previously (Denda *et al.*, 1998). All experiments were performed on 7- to 10-week-old male hairless mice (HR-1, Hoshino, Japan). The measurement of skin barrier function, disruption of the barrier, and application of the test sample were carried out under anesthesia with nembutal. For barrier recovery experiments, skin on both flanks was treated by repeated tape-stripping until the TEWL reached 7–10 mg cm^{-2} per hour, as described previously (Denda *et al.*, 1998). Immediately after tape-stripping, a solution of each reagent was applied on the skin (100 µl per one side of the flank). For aqueous solution, the skin surface treated with the solution was covered with a plastic wrap for 20 minutes to aid absorption. Two points were measured per flank and eight mice were used to evaluate the effects of each treatment. TEWL was measured at the same sites at 1, 3, 6, and 24 hours after barrier disruption. Barrier disruption was done between 0700 and 0800 hours and then the barrier recovery rate was evaluated immediately to avoid the effect of circadian rhythm (Denda and Tsuchiya, 2000b). The barrier recovery results are expressed as percent recovery, because of the day-to-day variations in the extent of barrier disruption. In each animal, the percentage recovery was calculated by the following formula: (TEWL immediately after barrier disruption–TEWL at indicated time point)/(TEWL immediately after barrier disruption—baseline TEWL) × 100%. In our case, TEWL immediately after barrier disruption was 7–10 mg cm^{-2} per hour, and the baseline TEWL (before tape-stripping) was 0.10–0.15 mg cm^{-2} per hour.

Statistics

Data are presented as the mean ± SD. The statistical significance of the differences between the two groups was determined by *t*-test. For more than two groups, significance was determined by analysis of variance and Tukey's test. *P*-values <0.05 were considered statistically significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signaling. *Nat Rev Mol Cell Biol* 1:11–21

Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 6:328–40

Celli A, Mackenzie DS, Crumrine DS *et al.* (2010) Endoplasmic reticulum Ca^{2+} depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis. *Br J Dermatol* 164:16–25

Cheng X, Jin J, Hu L *et al.* (2010) TRP channel regulates EGFR signaling in hair morphogenesis and skin barrier formation. *Cell* 141:331–43

Dabertrand F, Fritz N, Mironneau J *et al.* (2007) Role of RYR3 splice variants in calcium signaling in mouse nonpregnant and pregnant myometrium. *Am J Physiol Cell Physiol* 293:C848–54

Denda M, Denda S (2007a) Air-exposed keratinocytes exhibited intracellular oscillation. *Skin Res Technol* 13:195–201

Denda M, Fuziwara S, Inoue K (2003a) Influx of calcium and chloride ions into epidermal keratinocytes regulates exocytosis of epidermal lamellar bodies and skin permeability barrier homeostasis. *J Invest Dermatol* 121:362–7

Denda M, Fuziwara S, Inoue K (2003b) Beta-2-adrenergic receptor antagonist accelerates skin barrier recovery and reduces epidermal hyperplasia induced by barrier disruption. *J Invest Dermatol* 121:142–8

Denda M, Fuziwara S, Inoue K (2004) Association of cyclic AMP with permeability barrier homeostasis. *J Invest Dermatol* 122:140–6

Denda M, Hosoi J, Asida Y (2000a) Visual imaging of ion distribution in human epidermis. *Biochem Biophys Res Comm* 272:134–7

Denda M, Inoue K, Fuziwara S *et al.* (2002) P2X purinergic receptor antagonist accelerates skin barrier repair and prevents epidermal hyperplasia induced by skin barrier disruption. *J Invest Dermatol* 119:1034–40

Denda M, Nakatani M, Ikeyama K *et al.* (2007b) Epidermal keratinocytes as the forefront of the sensory system. *Exp Dermatol* 16:157–61

Denda M, Sato J, Masuda Y *et al.* (1998) Exposure to a dry environment enhances epidermal permeability barrier function. *J Invest Dermatol* 111:858–63

Denda M, Tsuchiya T (2000b) Barrier recovery rate varies time-dependently in human skin. *Br J Dermatol* 142:881–4

Ducreux S, Zorzato F, Müller C *et al.* (2004) Effect of ryanodine receptor mutations on interleukin-6 release and intracellular calcium homeostasis in human myotubes from malignant hyperthermia-susceptible individuals and patients affected by central core disease. *J Biol Chem* 279:43838–46

Elias PM, Ahn SK, Denda M *et al.* (2002) Modulations in epidermal calcium regulate the expression of differentiation-specific proteins. *J Invest Dermatol* 119:1128–36

Fuziwara S, Inoue K, Denda M (2003) NMDA-type glutamate receptor is associated with cutaneous barrier homeostasis. *J Invest Dermatol* 120:1023–9

Fuziwara S, Suzuki A, Inoue K *et al.* (2005) Dopamine D2-like receptor agonists accelerate barrier repair and inhibit the epidermal hyperplasia induced by barrier disruption. *J Invest Dermatol* 125:783–9

Jin D, Liu HX, Hirai H *et al.* (2007) CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* 446:41–5

Klegeris A, Choi HB, McLarnon JG *et al.* (2007) Functional ryanodine receptors are expressed by human microglia and THP-1 cells: their possible involvement in modulation of neurotoxicity. *J Neurosci Res* 85:2207–15

Kolly C, Suter MM, Muller EJ (2005) Proliferation, cell cycle exit, and onset of terminal differentiation in cultured keratinocytes: pre-programmed pathways in control of c-Myc and Notch1 prevail over extracellular calcium signals. *J Invest Dermatol* 124:1014–25

Kushnir A, Betzenhauser MJ, Marks AR (2010) Ryanodine receptor studies using genetically engineered mice. *FEBS Lett* 584:1956–65

Lauckner JE, Hille B, Mackie K (2005) The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc Natl Acad Sci USA* 102:19144–9

Mackrill JJ (2010) Ryanodine receptor calcium channels and their partners as drug targets. *Biochem Pharmacol* 79:1535–43

Mauro T, Bench G, Sidderas-Haddad E *et al.* (1998) Acute barrier perturbation abolishes the Ca^{2+} and K+ gradients in murine epidermis: quantitative measurement using PIXE. *J Invest Dermatol* 111: 1198–201

Medina-Ortiz WE, Gregg EV, Brun-Zinkernagel AM *et al.* (2007) Identification and functional distribution of intracellular Ca^{2+} channels in mouse lacrimal gland acinar cells. *Open Ophthalmol J* 1:8–16

Menon GK, Elias PM, Feingold KR (1994) Integrity of the permeability barrier is crucial for maintenance of the epidermal calcium gradient. *Br J Dermatol* 130:139–47

Sun TT, Green H (1976) Differentiation of the epidermal keratinocyte in cell culture: formation of the cornified envelope. *Cell* 9:511–21

Tsutsumi M, Kitahata H, Nakata S *et al.* (2010) Mathematical analysis of intercellular calcium propagation induced by ATP. *Skin Res Technol* 16:146–50

Thastrup O, Cullen PJ, Drobak BK *et al.* (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. *Proc Natl Acad Sci USA* 87:2466–70

Zucchi R, Ronca-Testoni S (1997) The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacol Rev* 49:1–51

Zvaritch E, Depreux F, Kraeva N *et al.* (2007) An $Ryr1^{I4895T}$ mutation abolishes Ca^{2+} release channel function and delays development in homozygous offspring of a mutant mouse line. *Proc Natl Acad Sci USA* 104:18537–42